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FOREWORD

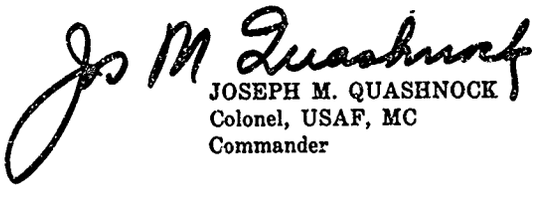
The experiments described in this report were conducted in the Pharmacology-Biochemistry Branch under task No. 775307. The work was performed between September 1965 and January 1966. The paper was submitted for publication on 15 May 1969.

The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences–National Research Council.

Decaborane used in the study was obtained from the Mann Research Labs. Inc., 136 Liberty St., New York, N.Y.

The authors express appreciation to Lieutenant Colonel Irving Davis, Colonel Harold L. Bitter, and the personnel of the Applied Physiology Branch for their encouragement and assistance. The special assistance of Major Harold Casey in interpretation of the histologic slides is gratefully acknowledged.

This report has been reviewed and is approved.



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Commander

ABSTRACT

The effect of decaborane (15 mg./kg.) on the hepatic glycogen of male rats maintained at altitude (18,000 ft.) under normoxic conditions was compared with the effect on similarly injected, ground level controls. Results showed that decaborane decreased the glycogen level of fed rats at ground level, but did not affect glycogen levels in the fed, altitude group. The hepatic glycogen level of fasted rats at altitude was the equivalent of that of fasted rats at ground level. These results indicate that the mechanism whereby decaborane produces a depletion of liver glycogen is altered under conditions of reduced pressure.

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I. INTRODUCTION

The boron hydrides possess certain characteristics which make them highly attractive candidates for use as solid propellants for rockets. Their effectiveness as reducing agents and their ability to react with a variety of chemicals (1) also raise the possibility that they can adversely affect biologic organisms. Decaborane ($B_{10}H_{14}$) is a solid borohydride which has been considered as a potential missile fuel; however, the toxicity of this compound presents a hazard to individuals who may be exposed to it.

Decaborane produces a myriad of toxic symptoms. A detailed review of the toxicology and pharmacology of this chemical has been prepared by Merritt (2). Few investigations have been made on the biochemical, physiologic, and pathologic changes induced by this compound, and nothing is known concerning the effects resulting from exposure to decaborane at altitude—a possible occurrence if this chemical were used as the fuel for a space vehicle.

This preliminary study was initiated to investigate the effects of decaborane on a specific metabolic system—liver glycogen. The experiment was designed so that measurement of any changes which occurred could be observed under different environmental conditions— namely, ground level and altitude.

II. METHODS AND MATERIALS

Male Sprague-Dawley rats weighing between 250 and 275 gm. were divided into groups of 6 rats each. Animals were allowed food and water ad libitum except where otherwise indicated.

Four groups of rats were placed in an altitude chamber and maintained at 18,000 ft. for 7 days in order to acclimate them to the decreased pressure. An atmosphere of 46% oxygen and 54% nitrogen was used within the chamber to eliminate the stress of hypoxia upon the rats. Under these conditions, the oxygen tension was equivalent to that present at sea level. Four groups of animals were maintained outside the chamber at ground level and used as ground controls. The chamber was entered each day to replenish water and food and to observe the animals. The rats were weighed daily. No significant difference was found between the average weight of the altitude rats and that of the ground level rats.

On the eighth day, the animals in the chamber were treated as follows: 2 groups were injected with 15 mg./kg. decaborane; the other 2 groups were injected with corn oil. One group of the decaborane-injected rats and 1 group of altitude controls were allowed access to water only, while the remaining 2 groups had free access to food and water.

The ground level groups were treated in the same manner: 2 groups were injected with decaborane; 2 groups with corn oil; 1 group of decaborane-injected animals and 1 of corn oil – injected rats were allowed water only; the other 2 groups were allowed both food and water.

Decaborane is relatively water-insoluble and was, therefore, dissolved in corn oil for injection. A dose of 15 mg./kg. body weight

was used for all of the experiments and the injections were made intraperitoneally.

Twenty-four hours after injection, the animals were sacrificed by a sharp blow on the back of the neck and the livers were removed. The tissue was immediately fixed in absolute alcohol. The tissue processor for light microscopy has a 3-step function: dehydration, clearing, and infiltration. Because glycogen is soluble in water, the dehydration step was omitted. After overnight fixation in absolute alcohol, the tissue was placed in chloroform instead of the standard alcohol series. The tissue was then embedded in paraffin blocks, and sections cut and mounted on glass slides. After deparaffination, the slides were stained with Best's carmine which colors the tissue glycogen a bright red. A detailed description of the technics can be found in standard texts (3, 4).

The slides were examined in a single-blind series by a pathologist, and the amount of glycogen present was graded on each sample on a relative basis from 0 to 4 plus (figs. 1—5).

III. RESULTS AND DISCUSSION

Comparison of comparable groups of altitude and ground level animals showed no differences in the amount of liver glycogen with the exception of the decaborane-injected, nonfasted group (table I). Comparison of glycogen levels in this group revealed a decrease in liver glycogen in the rats injected at ground level. In contrast, the group at altitude had a glycogen level comparable to that of the noninjected-nonfasted groups. These results indicate that decaborane is able to deplete hepatic glycogen in fed animals at ground level, but that this effect is absent at 18,000 ft.

Decaborane is known to produce hyperglycemia in animals and man as well as a reduction in liver glycogen (5). The glucose tolerance curve which is observed after administration of decaborane is similar to that seen in diabetes. Thus, results obtained with the nonfasted, decaborane-injected, ground level group are comparable to those reported in the literature.

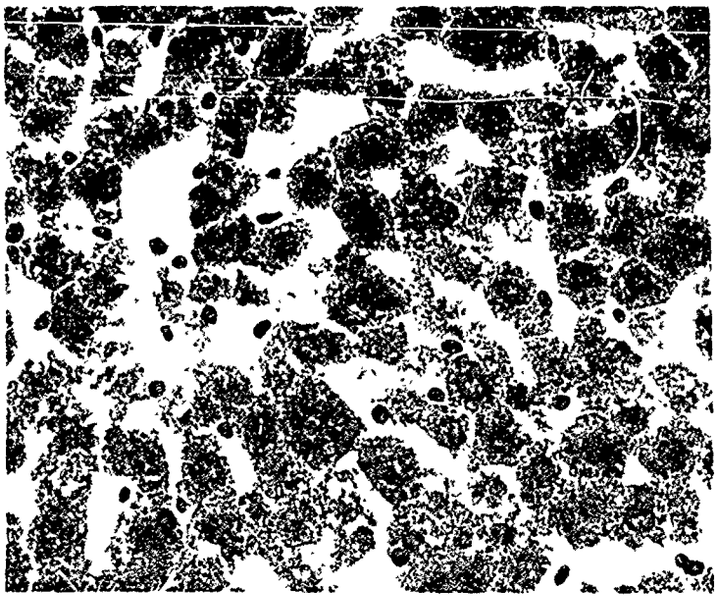

FIGURE 1

Liver sample taken at altitude from a fed, injected rat. (×400)

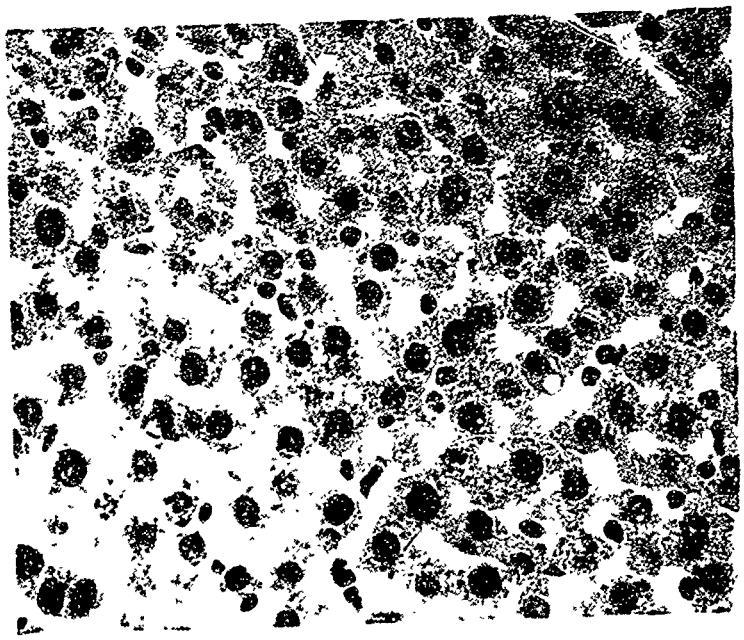

FIGURE 2

Liver sample taken at altitude from a fasting, injected rat. (×400)

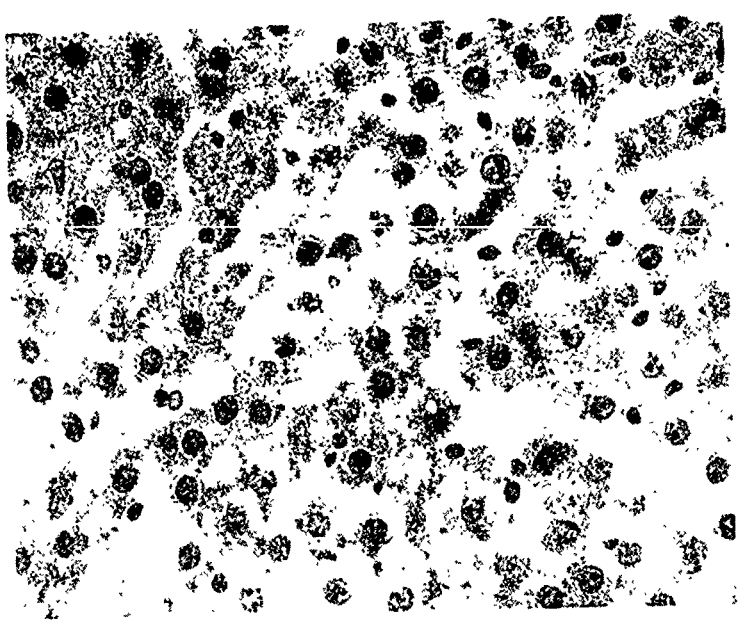

FIGURE 3

Liver sample taken at ground level from a fed, injected rat. (×400)

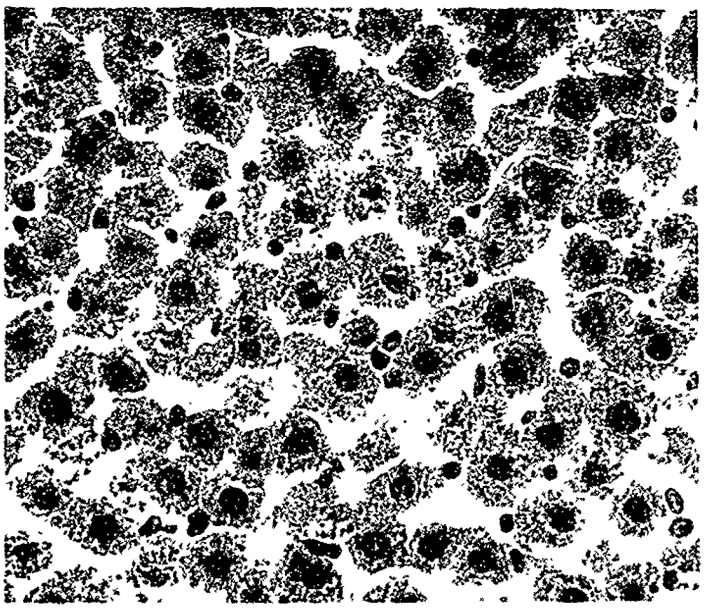

FIGURE 4

L ver sample taken at altitude from a fed, noninjected rat. (×400)

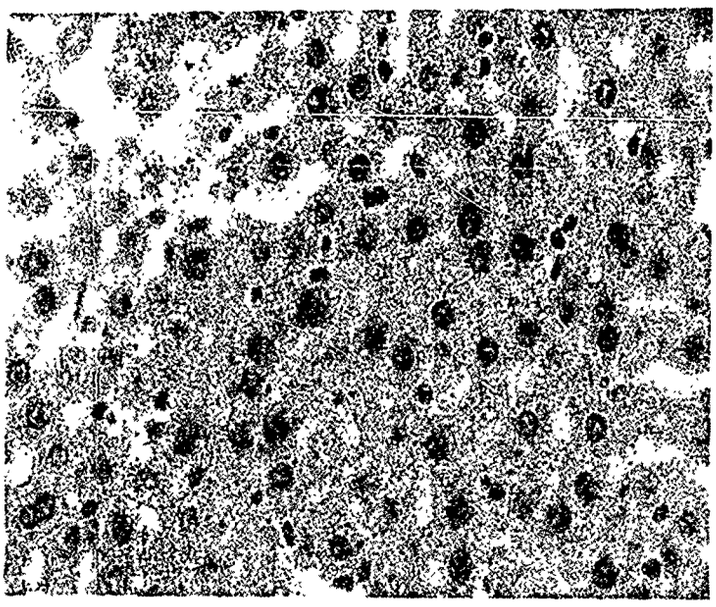

FIGURE 5

Liver sample taken at altitude from a fasting, noninjected rat. (×400)

TABLE I

*Liver glycogen content**

Animal group	Altitude	Ground level
Fasting, injected	+	+
Fasting, control	+	+
Fed, injected	++++	+
Fed, control	++++	++++

*Each specimen graded on a relative basis from 0 to 4 plus.

The lack of any effect on glycogen in the decaborane-injected, nonfasted group at altitude is difficult to explain. Decaborane is known to have some effect on the glycolytic pathway in rats, but if an interruption of glucose catabolism were the primary effect of this compound, then it would be logical to assume that an increase and not a depletion of glycogen would also have been seen in the rats injected at ground level. The increase of glycogen may be due to inhibition of glycogen phosphorylase, resulting in a cascade effect of enzymic reactions which leads to the hydrolysis of glycogen to glucose-1-phosphate. The failure of decaborane to produce glycogen depletion at altitude may be due to an inhibition of the breakdown of glycogen, and this mechanism may not be operative at ground level.

Differences have been reported in carbohydrate metabolism at altitude under normoxic conditions. Using dogs, Taub and Bernardini (6) observed an increase in the glucose tolerance curve at 27,000 ft. after 1 hour. Furthermore, the action, metabolism, and distribution of a drug may be affected by altitude. The glucose tolerance curve of dogs maintained at 27,000 ft. under normoxic conditions was altered by administration of amphetamine, meperidine, and diphenhydramine (6, 7, 8). Hexobarbital sleep time was decreased in mice exposed to 18,000 ft. for 5 days under ambient oxygen (9). The cited studies also revealed a difference in the distribution and the metabolism of hexobarbital. The foregoing reports show that it is possible that alteration of normal biochemical processes, as well as drug response, can combine to modify the effects which are normally seen at ground level. Any attempt to discuss the mechanism responsible for the difference between the effect of decaborane on liver glycogen at altitude and at ground level would be highly speculative, given the presently available information.

The experiments reported in this study do present two interesting facts: (1) a biochemical effect induced by a chemical can be altered by altitude; and (2) this alteration is due to the difference in reduced pressure and not to hypoxia.

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